International Food Research Journal 23(4): 1558-1563 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effects of drying methods, solvent extraction and particle size of Malaysian brown seaweed, *Sargassum* sp. on the total phenolic and free radical scavenging activity

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Article history

Received: 14 April 2015
Received in revised form: 22 December 2015
Accepted: 29 December 2015

Keywords

Sargassum sp.
Total polyphenol
Radical scavenging activity
Drying methods
Solvent type
Particle size

Abstract

This study was conducted to evaluate the effect of different drying methods, particle size and extraction solvent on the antioxidant properties of Malaysian brown seaweed *Sargassum* sp. Oven-, sun- and freeze-dried method were employed in this study and the obtained dried seaweed were passed through two sieve size of 2.00 mm and 0.25 mm prior to extraction with boiling water (infusion technique) and aqueous ethanol (50%). *Sargassum* sp. was evaluated for their total phenolic content (TPC) which were determined by spectrophotometry using Folin-Ciocalteu assay and expressed as gallic acid equivalent (GAE) in mg/g dry weight (dw) and free radical scavenging assay were used stable DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent. TPC and DPPH radical scavenging activity (RSA) showed a significant higher ($P < 0.05$) for oven dried samples with particle size of 0.25 mm in hot water extraction. A significant and positive high Pearson's correlations was observed between TPC and DPPH assay for particle size study ($r = 0.88$) and solvent extraction study ($r = 0.81$) which indicated that phenolics compound were main contributor of antioxidant activity in *Sargassum* sp. extracts. A strong free radical scavenging activity and higher phenolics contents in *Sargassum* sp. suggested that it has great potential in the food industry as a functional food ingredient.

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Introduction

Brown seaweeds are rich in bioactive compounds that could be exploited as functional ingredients for both human and animal health applications. The genus *Sargassum*, a kind of brown algae belong to the class Phaeophyceae have been reported to have a wide range of bioactive activities (Marimuthu *et al*., 2012) such as antioxidant, anti-inflammatory, anti-cancer and anti-diabetic (Wijesinghe and Jeon, 2012). It belongs to the family Sargassaceae and order Fucales. *Sargassum* sp. can be found growing naturally in Pulau Bum Bum, Semporna, Sabah. Three edible brown seaweeds from North Borneo, Malaysia (*Dictyota dichotoma, Sargassum polycystum* and *Padina* sp.) had been studied by Patricia *et al.* (2008) and showed good antioxidant capacity. This indicated that brown seaweed can be a good source of natural antioxidant that can be used as an ingredient in food and beverages product.

Extracting antioxidants from plant material most often involves the methods of extraction. The choice of solvent has been shown to have a significant influence on the concentration of antioxidants extracted (Anwar *et al*., 2013). Attention is now being directed to the extraction techniques that rely on solvents that are not hazardous to human health (Durling *et al*., 2007). Antioxidant activity seemed to be influenced by the drying procedure prior to extraction. Chan *et al.* (2008) reported that all thermal drying methods tested (microwave-, sun- and oven-drying) resulted in a decrease in the total polyphenol content (TPC) of leaves and ginger tea. Rhim *et al*. (2009) studied the effect of different drying techniques on the antioxidant activity of the herb also concluded that most forms of drying (including air-, hot air-, sun- and freeze-drying) had adverse effects on antioxidant activity.

Therefore, when attempting to identify potential sources of natural antioxidants, in addition to targeting plants high in antioxidant activity, it is also wise to consider extraction and drying parameters. The efficiency of solid/liquid extraction processes is affected by critical processing parameters, such as temperature, nature of solvent, structure of solid matrix (mainly particle size) and extraction time

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(Maria *et al*., 2009). This means that each plant matrix/extraction solvent pair behave es in a unique way, so it should be studied as such. The aim of this study was to evaluates the effect of different drying method (oven, sun and freeze drying) prior to extraction on the TPC and radical scavenging activity (RSA) of *Sargassum* sp. as well as the influence of the particle size and type of solvent.

Materials and Methods

Chemical and reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl) and gallic acid were purchased from Sigma-Aldrich (M) Sdn Bhd Chemicals. Sodium carbonate, Folin-Ciocalteau reagent, ethanol and methanol (analytical grade) were purchased from Merck (Germany).

Sample preparation

Malaysian brown seaweed, *Sargassum* sp. was obtained from Perusahaan Rumpai Laut Juni Kg. Singgamata, Pulau Bum-Bum, Semporna, Sabah. Fresh brown seaweeds were sun dried prior transported to Peninsular Malaysia to avoid spoilage. The sun dried brown seaweed was soaked in the water in order to remove all the epyphites including salt and sand that attached to the surface. The sample was washed thoroughly with tap water and drained followed by drying.

Drying of samples

The frozen seaweeds were thawed and cut manually with scissors into a uniform size (~1 cm). The chopped seaweed was divided into three portions (100 g each) and subjected to three different drying processes. The first portion was oven-dried at 50°C (for 1 day), the second portion was sun-dried (for 3 days) and the remaining portion was freeze-dried for 5 days. All the dried samples for each drying methods were ground by using a coffee mill, set at 7 for about 1 min in order to obtain corresponding powder before passed through two sieve sizes by using Ultra Centrifugal Mill (Retsch ZM200) to obtain seaweed dried powder (SDP) with particle size of 0.25 mm and 2.0mm. The obtained SDP were then used for extraction of antioxidant.

Extraction of samples

To study the effect of different particle size, the antioxidants were extracted from SDP (~1.0 g) using 100% distilled water (20 mL) for both particle size of ground powder (2.0 and 0.25 mm). Meanwhile, to study the effect of different solvent extraction, SDP were extracted using ethanol and water at the ratio of 50:50), and the second extraction was infused the in 20 mL of hot water. All the extraction was performed by an orbital shaker for 24 hrs at room temperature and then the extracts filtered through filter paper (Whatman No. 4). The extracts were assayed for their TPC and RSA, as described below. All experiments were run in triplicate.

Determination of total phenolic content (TPC)

The TPC in the extract was determined using Folin-Ciocalteau reagent (Singleton and Rossi 1965) with some modifications. Fresh weight of each samples were converted into dry weights on the basis of the moisture content. Each of the extract (100 µl) was transferred into a test tube and then mixed thoroughly with 0.5 ml Folin-Ciocalteau reagent (prediluted 10-fold with distilled water). After mixing for 5 min, 1.0 ml of 7.5% (w/v) sodium carbonate was added. The mixtures were agitated with a vortex mixer, and then allowed to stand in the dark for a further 120 min at ambient temperature. The absorbance of the extracts and a prepared blank were measured at 765 nm using a spectrophotometer (UV-vis model 50 Probe, Varian Cary). The TPC was expressed as mg gallic acid equivalent (GAE)/g dry weight which was determined from known concentrations of gallic acid standard prepared similarly. Data were reported as a mean ± standard deviation for three replications.

Determination of free radical scavenging activity

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometric assay uses stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent, according to a slightly modified method of Blois (1958). 100 µl of the extracts was added to 2.9 ml of a 0.004% methanol solution of DPPH. After a 120 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The percentage of inhibition of free radical DPPH by the extracts was calculated as follow:

$$\text{Inhibition (\%)} = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

Statistical analysis

Each of the measurements described above was conducted in triplicate and the mean data ± SD

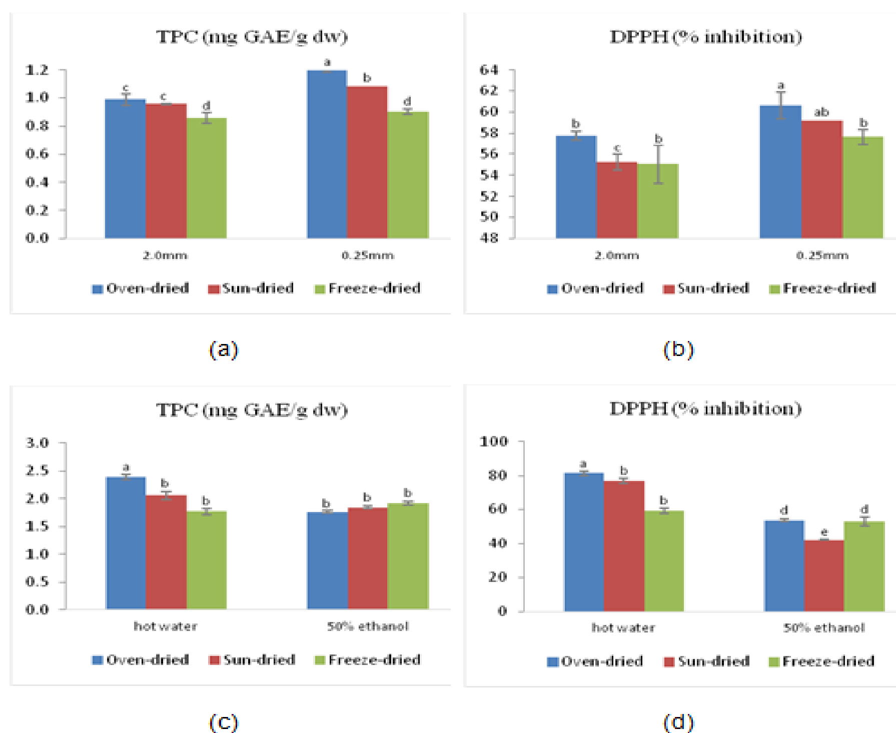

Figure 3. Effect of different drying methods on TPC and DPPH RSA of *Sargassum* sp. with different particles size (a and b) and solvent extractions (c and d). Different letters on the bars show significant difference ($p < 0.05$). Each bar is mean ± standard deviation of 3 measurement.

(standard deviation) were reported. The data collected were statistically analysed using the Statistical Analysis Software (SAS) package (version 9.1.2 of SAS Institute, Inc. Cary, NC, 2008). Statistically significant differences ($P < 0.05$) in the antioxidant properties of the samples were determined by one way analysis of variance (ANOVA). Duncan Multiple Range Test (DMRT) was used to determine significant differences between the means.

Results and Discussions

Effect of different drying methods on TPC and DPPH

The TPC of air-dried, sun-dried and oven-dried *Sargassum* sp. varied significantly ($P < 0.05$) with the oven-dried samples having the highest phenolic contents for both particle size (2.00 mm and 0.25 mm) (Figure 3a) and extracting solvent (hot water) (Figure 3c). This effect of drying method on TPC also shows similar result for DPPH radical scavenging assay. Drying samples using an oven at 50°C have shown aggressively drying method for *Sargassum* sp. which consistently produced SDP extracts high in antioxidants (TPC and DPPH RSA) regardless of the particle size and extraction solvents used, except for TPC value of freeze-dried samples when extracting with 50% ethanol (Figure 3c). Both of particle size (2.00 and 0.25 mm) and extraction solvents used (hot water and aqueous ethanol) exhibited high value for TPC and DPPH assay. Meanwhile, the least aggressive drying method, freeze-drying has produced extracts low in TPC and DPPH RSA which dependent of particle size (Figure 3a and 3b) but independent when extracting with 50% ethanol (Figure 3c and 3d).

As reported by Mrkic *et al.* (2006), the importance of a short drying time to maximise antioxidant activity of broccoli extracts seems to be an important factor in this study also. In this study, different drying length have been conducted depends on the method used to ensure the fresh samples were completely dry. The oven-dried samples had been dried for 1 day, the sun-dried samples for 3 days while the freeze-dried for 5 days indicating that as the length of drying time increased, antioxidant activity decreased.

Several studies had been focused on the use of higher temperatures (95°C-120°C) for drying prior to extraction. Many studies have reported losses in TPC and antioxidant activity (AOA) of plant samples following thermal treatments. Losses were mainly reported in vegetables (Ismail *et al.*, 2004 and Roy *et al.*, 2007). Whereas, increase in AOA following thermal treatment has been reported in tomato (Dewanto *et al.*, 2002), and Shiitake mushroom (Choi *et al.*, 2006). From this study, oven-drying at 50°C is superior drying process for *Sargassum* sp. and will be chosen for drying method for the next analysis. The point at which the temperature begins to significantly

Table 1. TPC and DPPH RSA of SDP (*Sargassum* sp.) extracts obtained using different particle sizes and drying processes

Assay	TPC (mg GAE/g dry weight)		DPPH (% inhibition)	
Particle size	2.0 mm	0.25 mm	2.0 mm	0.25 mm
Oven-dried	0.9889 ± 0.0418^c	1.1972 ± 0.0139^a	57.78 ± 0.40^b	60.65± 1.26^a
Sun-dried	0.9559 ± 0.0029^c	1.0845 ± 0.0030^b	55.23± 0.76^c	59.19± 0.79^{ab}
Freeze-dried	0.8601 ± 0.0387^d	0.9024 ± 0.0167^d	55.04± 1.79^c	57.64± 0.70^b

Data was expressed as mean ± SD, each value is a mean of triplicate reading. Means with the same letter are not significantly different ($p < 0.05$)

and adversely affect antioxidant activity will be study for the next experiment through optimization technique.

Effect of particle size on TPC and DPPH RSA

To study the effect of particle size, dried seaweed from each drying methods were passed through two sieve size to produce seaweed dried powder (SDP) with particles size of 2.00 mm and 0.25 mm. The TPC and DPPH radical scavenging activity (RSA) of the SDP extracts for three drying methods and two particle size are shown in Table 1. Result clearly showed that SDP with particle size of 0.25 mm (oven-dried) give the highest TPC (1.1972 mg GAE/g dry weight) and DPPH RSA (60.65%) which significantly different ($P < 0.05$) from other extracts. Overall results show that SDP with particle size of 0.25 mm give the highest TPC and percentage inhibition of DPPH compared to SDP with particle size of 2.00 mm.

As can be observed, the phenolic contents increased with decreasing particle size, this means that an increase in the surface area available for molecular transport contribute to a more extensive mass transfer of solutes between phases (Maria *et al*., 2009). Presumably, when the particle size is reduced, the accessible surface is increased, resulting in the observed enhancement of the extraction efficiency, except for freeze-dried method. The positive effect of reducing the particle size have been previously reported in other studies about phenolic extraction; it has given very positive results in other plants materials like black currant pomace and black cohosh (Landho and Meyer, 2001). Hence, the smaller the particle size of seaweed dried powder, the higher the extraction efficiency and thus the higher the phenol concentration and radical scavenging activity of the resulting extracts.

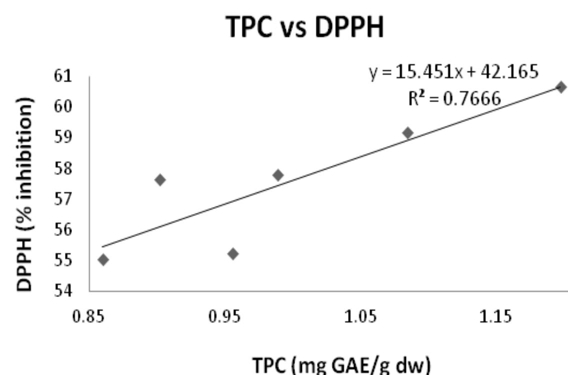

Figure 1. Plot of TPC versus DPPH radical scavenging (varying in drying methods and particle size)

A plot of TPC versus percentage inhibition of DPPH for all the SDP extracts with particle size of 2.00 mm and 0.25 mm (Figure 1) exhibited a strong correlation ($r = 0.88$), indicating that the results of both TPC and DPPH RSA strongly corresponded to each other. Meanwhile, coefficient of determination (R^2) was measured on how well the regression line represents the data which shows the association between TPC and DPPH assay ($R^2 = 0.7666$). The results presented in this study indicate that high antioxidant activity is associated with a high phenolics content so it could be argued that the only virtue in performing the TPC assay would be as a screen to evaluate extracts further by the DPPH assays (Clarke *et al*., 2013).

Effect of solvent extraction on TPC and DPPH RSA

From our study on the effect of particle size on TPC and DPPH RSA, SDP with particle size of 0.25 mm have shown to give the highest value for both assay. Therefore in this study, we have chosen SDP with particle size of 0.25 mm in order to evaluate the effect of solvent extraction on TPC and DPPH RSA assay.

To study the effect of solvent extraction, SDP which obtained from three different drying method (oven-, sun- and freeze-dried) were extracted with hot water (infusion technique) and aqueous ethanol (50%) and result obtained were summarized in Table 2. As can be observed from Table 2, extraction with hot water resulted in highest value for TPC (2.3961 mg GAE/g dry weight) for SDP obtained by oven-dried method. There is no significant different ($P < 0.05$) for TPC in other SDP extracts which ranging from 1.7647–2.0546 mg GAE/g dry weight both for hot water and aqueous ethanol extraction. Similar to TPC, the oven dried samples extracted with hot water was also exhibited the highest inhibition of RSA

Table 2. TPC and DPPH RSA of SDP (*Sargassum* sp.) extracts obtained using different solvent extraction and drying processes

Assay	TPC (mg GAE/g dry weight)		DPPH (% inhibition)	
Solvent type	Hot water	50% ethanol	Hot water	50% ethanol
Oven-dried	2.3961 ± 0.0493[a]	1.7647 ± 0.0181[b]	81.37 ± 1.36[a]	53.75 ± 0.92[d]
Sun-dried	2.0546 ± 0.0703[b]	1.8392 ± 0.0230[b]	77.07± 1.50[b]	42.22± 0.21[e]
Freeze-dried	1.7699 ± 0.0596[b]	1.9150 ± 0.0266[b]	59.20± 1.72[c]	52.85± 2.53[d]

Data was expressed as mean ± SD, each value is a mean of triplicate reading. Means with the same letter are not significantly different ($p < 0.05$)

(81.37%). From this study, hot water was superior in their ability to extract antioxidant from SDP and was significantly more efficient than aqueous ethanol.

The suitable solvent for extracting target compounds should be selected carefully because the extracted compound will be based on the type of solvents used (Zarnowski and Suzuki, 2004). Ethanol and water are widely used solvents due to their low toxicity and high extraction yield and in advances their polarity can be modulated by mix them at selected ratio (Franco *et al*., 2008).

Figure 2 show correlation between TPC and DPPH assay of SDP extracts which obtained from different drying method (oven-, sun and freeze-dried) and extracting with hot water and aqueous ethanol. The results also demonstrated highly positive correlation coefficient between TPC and DPPH RSA of *Sargassum* sp. (r = 0.81), and coefficient of determination (R^2= 0.6579) from the plot graph indicated on how well the regression line represents the data which shows the association between TPC and DPPH assay. This support the aspects that the hot water extracts of *Sargassum* sp. with higher TPC have also higher percentage of inhibition by DPPH and thus superior antioxidant activity.

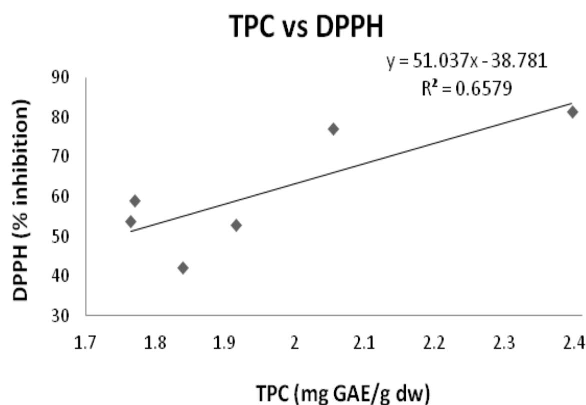

Figure 2. Plot of TPC versus DPPH radical scavenging (varying in drying methods and extracting solvent)

Conclusions

This study indicated that different drying methods, extraction solvent and particle size of material has a significant influence on antioxidant property of *Sargassum* sp. extracts. The highest TPC and percentage inhibition in DPPH was shown by oven drying methods. Water extracts (hot) showed better DPPH RSA and more phenol content as compared to aqueous ethanol (50% ethanol). Particle size was found to have significant influence on the phenol release, the latter increasing when smaller particle sizes are employed. From this study, it can be concluded that SDP with particle size 0.25 mm resulted in higher TPC and DPPH RSA compared to particle size 2.00 mm. Hence, oven drying at 50°C is the best drying process for *Sargassum* sp. and smaller particle size (0.25 mm) can extract more antioxidant component by using hot water as extraction solvent.

Acknowledgements

The author acknowledges with gratitude the financial support given by Malaysia Agriculture Research and Development Institute (MARDI) and special thanks to the Seaweed Downstream Research Centre (SDRC) and School of Chemical Sciences and Food Technology, Faculty of Science and Technology, UKM for this opportunity.

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